Experimental Technique for Optimizing Aerosolized Vaccine Efficacy

by

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ABSTRACT

Vaccination via aerosol has been proven to be as safe, as effective, and more appropriate for transportation when compared with vaccination via injection. These advantages make aerosolized vaccinations a realistic alternative to traditional injection vaccines for the developing world, where cold chain systems are impractical and the use of hypodermic needles can be unsafe. However, more research is needed to determine optimal parameters for vaccine aerosolization. This thesis presents an experimental setup to test Aerovax, a device designed to deliver aerosolized vaccinations in developing regions of the world. The experimental technique is the first effort to optimize vaccine aerosols across multiple variables, including input pressure, nebulizer geometry, and vaccine reconstitution. The setup provides a pressure input, sensors for ambient properties, and a device to measure particle size distribution.

Thesis Supervisor: Sanjay Sarma
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1. Introduction

Aerosolized vaccination is seen as a viable and necessary replacement for traditional injection vaccines in the developing world. Currently, subcutaneous injection requires the execution of a cold chain system[1] and the use of sterile hypodermic needles, both of which present a challenge to much of the world's poor.

A major barrier to vaccine delivery in developing regions lies in maintaining designated storage temperatures. The excessive warming or cooling of a vaccine will cause it to lose potency, rendering it useless. Transportation to rural areas presents a logistical challenge because it is difficult to keep vaccines at their appropriate temperatures for the entire journey. Successful delivery relies on absolute compliance with the cold chain system, which sets standards for personnel, equipment, and procedures to be followed[1].

Reduction of injections in the developing world would greatly benefit patients seeking immunization, as needle misuse is a common practice among healthcare workers. An inadequate number of biohazard waste facilities hinders safe disposal of used needles[2]. Consequently, used injection equipment is very often sold, reused, or recycled because of its commercial value, instead of being disposed of properly. Since 2003, the World Health Organization has encouraged the use of auto-disable syringes in all countries and has stressed that standard disposable syringes no longer be used for immunization[2]. However, as long as vaccines can be administered only by subcutaneous injection, the reuse and misuse of needles will likely continue.

The cold chain can be eliminated with the implementation of aerosolized vaccinations, as they would be delivered in a freeze-dried, powder form with no

temperature restrictions[3]. The implementation of aerosolized vaccines would remove the need for both cold supply chains and hypodermic needles, thus serving the developing world efficiently and safely. However, aerosolized vaccine technology is still under development and being tested for use.

Our lab is currently developing a new strategy called Aerovax, an inexpensive and portable device that will provide aerosolized immunizations to healthcare's last mile[4]. Once a vaccine has reached its destination, its powder form can be aerosolized for safe delivery to the patient.

A case example of the need for aerosolized vaccines is illustrated by the measles epidemic. An estimated 750,000 children die from measles every year, making it one of the most fatal respiratory diseases in children. With proper immunization, measles is entirely preventable[5]. For this reason, measles will be the first vaccine optimized for aerosolization by Aerovax. Vaccination via aerosol offers a tremendous opportunity for mass measles immunization; it has been found to vaccinate as successfully as subcutaneous injection[5] and can be administered to a wider geographical range.

The current status of aerosolized vaccination technology requires the development of vaccine powders that maintain both their potency and a particle size that is deliverable to the lungs via a nebulizer. The target particle size ranges from 3 μm to 5 μm. Particle sizes too small have the ability to penetrate the lungs, but are not large enough to carry a significant amount of vaccine; particle sizes too large carry a potent vaccine, but cannot gain access to the lungs to successfully vaccinate a patient. The different sizes of vaccine particles created by nebulization are referred to as the "particle size distribution," which

is a key parameter in the measurement of an aerosol for delivery to the respiratory system.

This thesis aims to develop a means to measure the efficacy of powder vaccines by developing an experimental technique to evaluate how powders respond to nebulization. With this technique, we can define an "aerosol fingerprint"[6] for a given vaccine to be aerosolized, which will classify the optimal conditions for effective vaccine delivery based on particle size distribution. Aerosolized vaccine particle distribution is dependent upon input nebulizer pressure, ambient temperature, relative humidity, nebulizer geometry, and vaccine reconstitution. The experimental setup built for this thesis to measure particle size distribution, based on the factors just listed, is described in the following section.

2. Experimental Setup

In the experimental setup, shown schematically in Figure 1, sensors measure ambient temperature, ambient relative humidity, and nebulizer temperature fluctuations. A transducer allows for regulation of the input pressure to the nebulizer. There is also a laser system that measures particle size distribution.

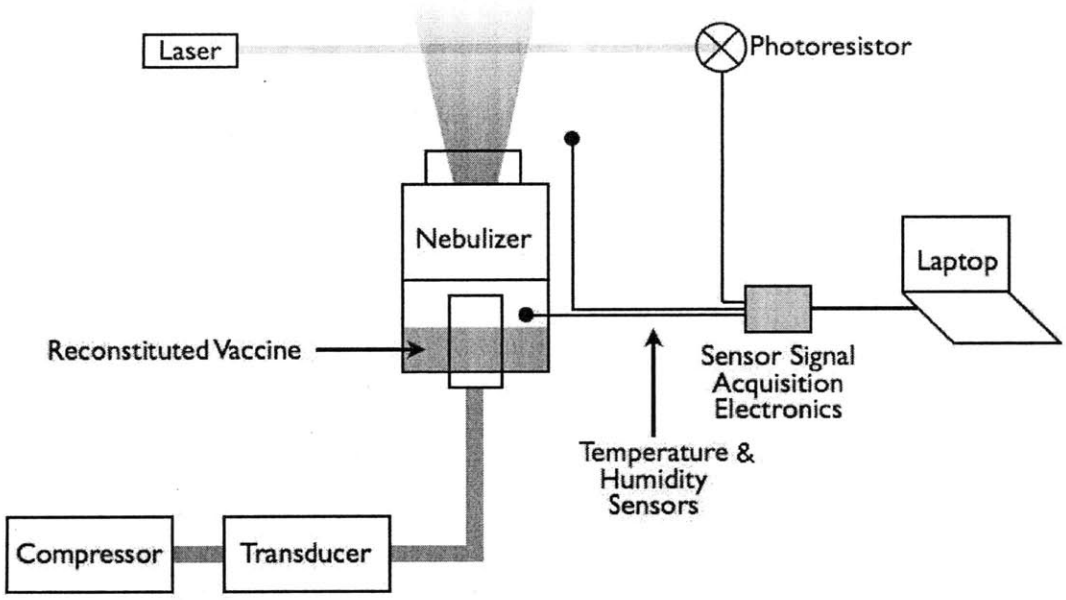

Figure 1. Schematic of the Aerovax experimental setup.

While individual parameters affecting aerosolized vaccination delivery have been studied in various experiments, this thesis is the first attempt to optimize across all significant variables.

2.1 Pressure

The pressure provided to the nebulizer determines the output flow rate of the aerosol and is hypothesized to have an effect on the particle size distribution of the vaccine. A higher flow rate will ensure less particle deposition in the nebulizer. As recommended[5, 6], pressure values ranging between 20 psi and 70 psi will be tested. As the main input for Aerovax, pressure plays an important role in the optimization of the other parameters.

It is important to note that while pressure can be input from a source that runs on electricity, it can also be delivered via a battery powered source, compressed air, or a human powered source, such as a foot pump. This feature is incredibly valuable in settings where either unreliable or no electricity is available, which is the case for Aerovax's target locations.

Figure 2 shows the Craftsman 12 V air compressor that supplies pressure to the system. It outputs 250 psi, which is much greater than the 20 psi to 70 psi required for nebulization.

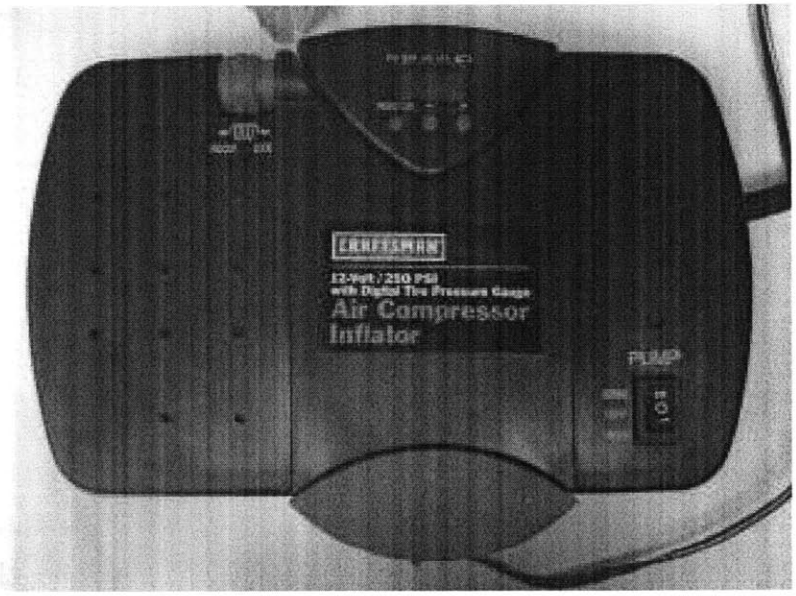

Figure 2. The compressor that supplies pressure to the setup.

A transducer, shown in Figure 3, is used to regulate the pressure through an input voltage of 0 V to 10 V. A higher voltage input to the transducer will result in a higher pressure output, with an approximate 27 psi maximum. A low voltage input will output a minimum pressure of 3 psi to the nebulizer.

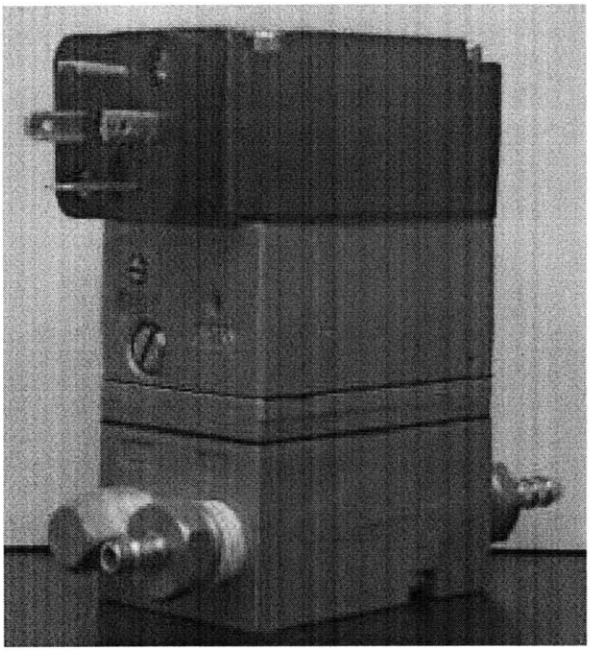

Figure 3. The transducer regulates pressure input from the compressor and creates the desired output to the nebulizer for vaccine aerosolization.

In the experimental setup, the transducer is located between the compressor and the nebulizer, as shown in Figure 1, with tubing connecting it to each. It is important to note that the transducer will only function with a pressure input and an output line that is not exposed to atmospheric pressure.

Figure 4 shows the configuration that was determined to be the most successful for regulating the pressure input. The power source chosen has terminals to connect wires to from the transducer, as well as 12 V cigarette lighter receptacle. This is a suitable match for the compressor, as it was originally intended for automobile use and has a cigarette lighter adapter.

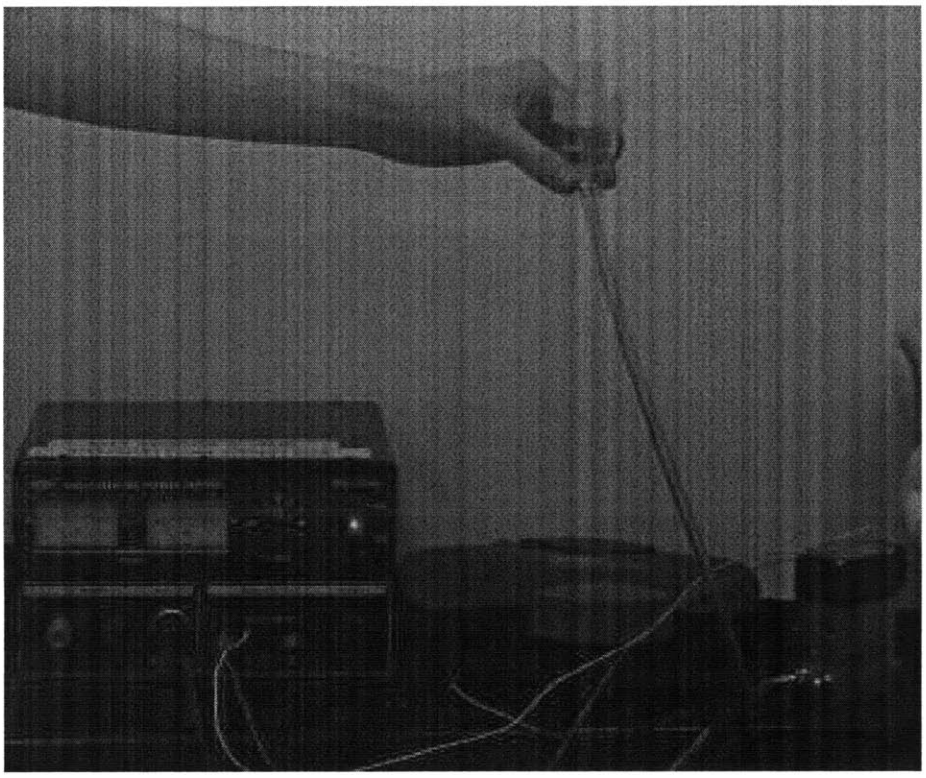

Figure 4. Setup of pressure input components.

2.2 Nebulizer Temperature

During aerosolization, the solution in the nebulizer cools and concentrates. Most of the heat loss is caused by evaporation of the nebulizer solution to saturate the gas used to generate the aerosol, while some cooling is caused by adiabatic expansion of the gas being generated[7]. Phipps, et al. found that the temperature of a solution aerosolized through a jet nebulizer at a low flow rate can be expected to decrease 5 to 6°C below the ambient temperature[7]. Nebulizers can be expected to cause a decrease of different temperature amounts, depending on their shape. A solution aerosolized at a high flow rate (8L/min, for example) can be expected to decrease 11°C to 15°C. The temperature change will typically occur within the first four minutes of aerosolization. This

significant temperature decrease can lead to an overall reduction in the particle size distribution, making it an important parameter to monitor[7].

The temperature change that occurs inside the nebulizer is measured with a Flexible Hermetic Sealed PFA RTD Sensor Probe, shown in Figure 5. The probe can be easily inserted into the head of the nebulizer and kept there throughout the entire experiment without disrupting the setup, allowing for real-time measurements. The four-wire configuration of the sensor decreases resistance from the wires and allows for easy integration with data-logging software[6].

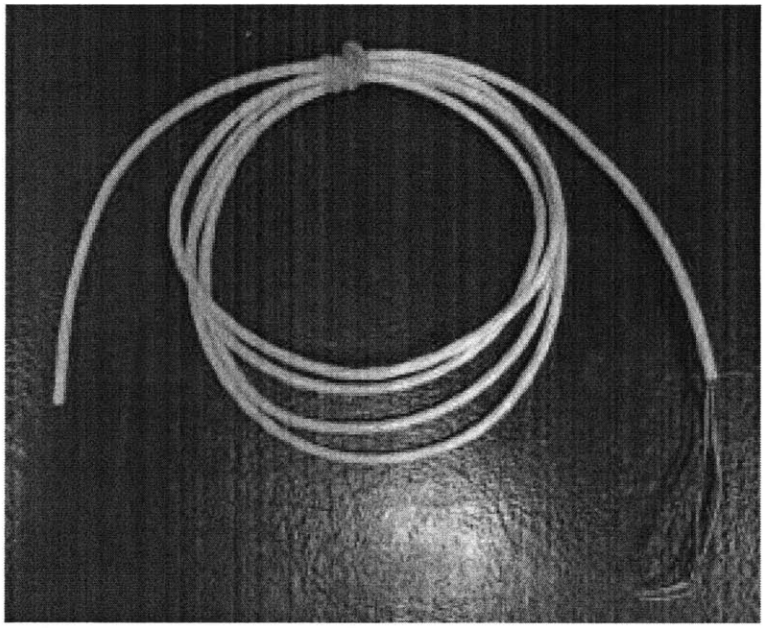

Figure 5. The Flexible Hermetic Sealed PFA RTD Sensor Probe, used to measure both nebulizer and ambient temperature.

2.3 Ambient Temperature and Relative Humidity

Aerovax will be expected to operate in a wide variety of climates throughout the developing world. In the field, ambient properties will be impossible to control;

however, it is important to observe the influence they exert, if any, on other parameters in the setup. The measurements taken will act as a gauge for how ambient properties might possibly alter the particle size distribution.

Relative humidity, for example, has the potential to cause post-aerosolization clumping[6]. It will be important to determine at what value and temperature this occurs, if at all, as clumping can lead to a larger particle size measurement.

The ambient temperature is measured with the same probe as the nebulizer temperature, shown in Figure 5. The measurement is taken before the experiment is started; the probe is then relocated to the head of the nebulizer.

The relative humidity is measured with a Honeywell HIH-4000 integrated circuit humidity sensor[6]. The small size of the sensor allows it to be easily attached to the existing particle size distribution measurement hookup, while remaining unobtrusive. It can register relative humidity values between 0% and 100%, making it appropriate for any possible climate simulation. While ambient properties are not possible to manipulate in the field, delivery times, pressure, and vaccine constitution parameters can be adjusted to minimize the effects of relative humidity and local temperature for a given region.

2.4 Particle Size Distribution

The size of the aerosolized particles is arguably the most important parameter to ensuring successful vaccination of the patient[5]. In vivo studies in adults have suggested a maximum particle size of 5 μm, while studies in children 4 years and younger suggest a cutoff of 4 μm. There is little in vivo data available for infants; consensus is that the appropriate maximum particle size is 4 μm or less[5].

Particles classified as too large (greater than 5 μm) will be unable to infiltrate below the vocal chords in an adult to the correct location for vaccine delivery, even though they carry a substantial amount of vaccine. Conversely, particles that are too small (less than 1 μm) will have the ability to penetrate and disperse within the lungs, but will not carry enough drug to effectively vaccinate the patient[5]. Trials completed with the Aerovax experimental setup will use these predetermined particle sizes as a guideline.

Nicholson indicated that measurement of the Aerovax particle size distribution was to be completed with Malvern Spraytec Laser Diffraction instrument[6]. For the purposes of this experiment, a device was constructed that measures the same qualitative properties as the Malvern Spraytec instrument, shown in Figure 6.

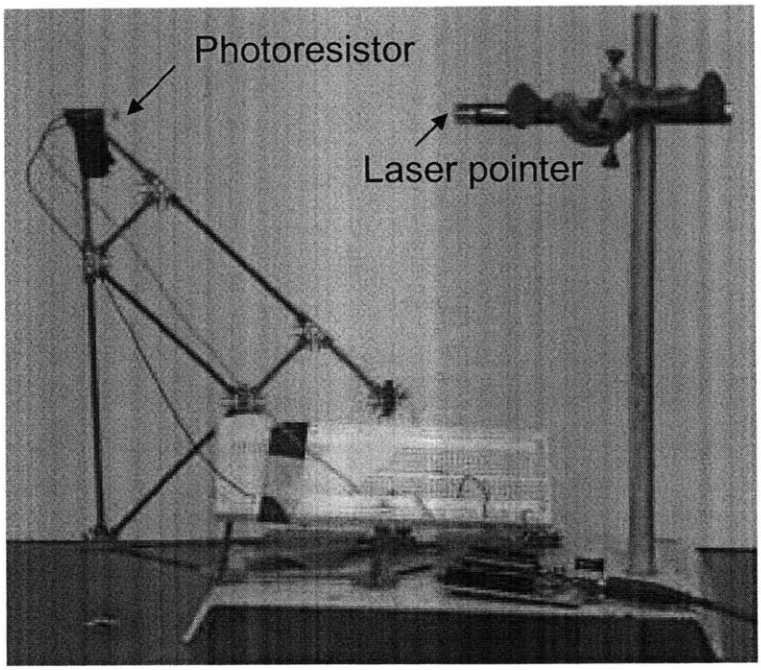

Figure 6. Device to measure particle size distribution. The laser pointer is aimed at the photoresistor and the nebulizer is held under the beam. The aerosol particles interrupt the light, and the amount of light blocked is measured.

A laser pointer is aimed at a photoresistor, which measures a constant light intensity when there are no particles present. The nebulizer is held under the laser beam and the vaccine (or for basic experimental purposes, water) is aerosolized, interrupting the constant beam of light, as shown in Figure 7.

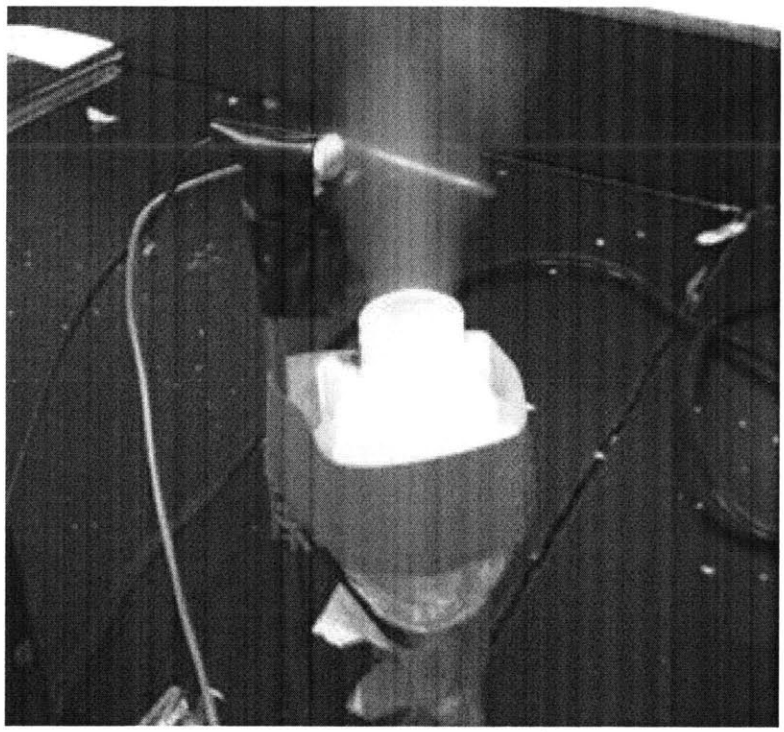

Figure 7. Aerosol being produced during nebulization. It interrupts the constant beam of light aimed at the photoresistor, and the resistor measures how much of the beam is blocked by the particles.

Smaller particles block less light than larger particles, showing a relationship between particle size and the amount of light allowed through to the resistor. This relationship can eventually be used to determine the actual size of the aerosolized particles.

The output from the device is a voltage that corresponds to particle size. The current setup allows for qualitative, but not quantitative, measurement; the particle sizes

can be compared to each other, but their actual sizes still need to be calibrated for quantitative measures. During experiments, the target particle sizes will be between 3 μm and 5 μm.

3. Results

3.1 Pressure

With an input of 12 V, the compressor output was 250 psi. The transducer decreased this pressure to a minimum of 3 psi with an input of 0 V. With a 10 V input, the maximum pressure output by the transducer was 25 psi, not the expected maximum of 27 psi. However, 25 psi was sufficient for the nebulizer to produce a visible aerosol, shown in Figure 7, that was detectable by the photoresistor. In the next setup, the transducer will be replaced by a model with which a higher range of pressures can be output. This will make the range of pressure available to the system more inclusive of all possible pressure values for aerosolization.

3.2 Ambient Temperature and Relative Humidity

A first order approximation of the relative humidity measured by the sensor can be determined by the equation

$$V_{out} = V_{\sup ply}((0.0062 \cdot SensorRH) + 0.16) \quad (1)$$

where V_{out} is the voltage output by the sensor, V_{supply} is the 5 V supplied to the sensor, and *Sensor RH* is the relative humidity to be solved for[8].

The temperature sensor was successfully attached to the system and will be able to record temperature measurements in future experiments.

3.3 Particle Size Distribution

Figure 8 shows a trial of the particle size detector measuring the particles in a water aerosol produced by pressure input with the use of a foot pump. The relationship depicted is between the resistance of the photodetector, which correlates to light amplitude, and time. The resistance was measured by the voltage across a resistance-based voltage divider. The peaks in the graph depict when no aerosol is present and the detector is picking up the uninterrupted light beam of the laser. The troughs show when a strong surge of aerosol is interrupting the laser beam. The drift observed in the graph was a result of the manual setup and was not observed in future iterations with steady pressure inputs.

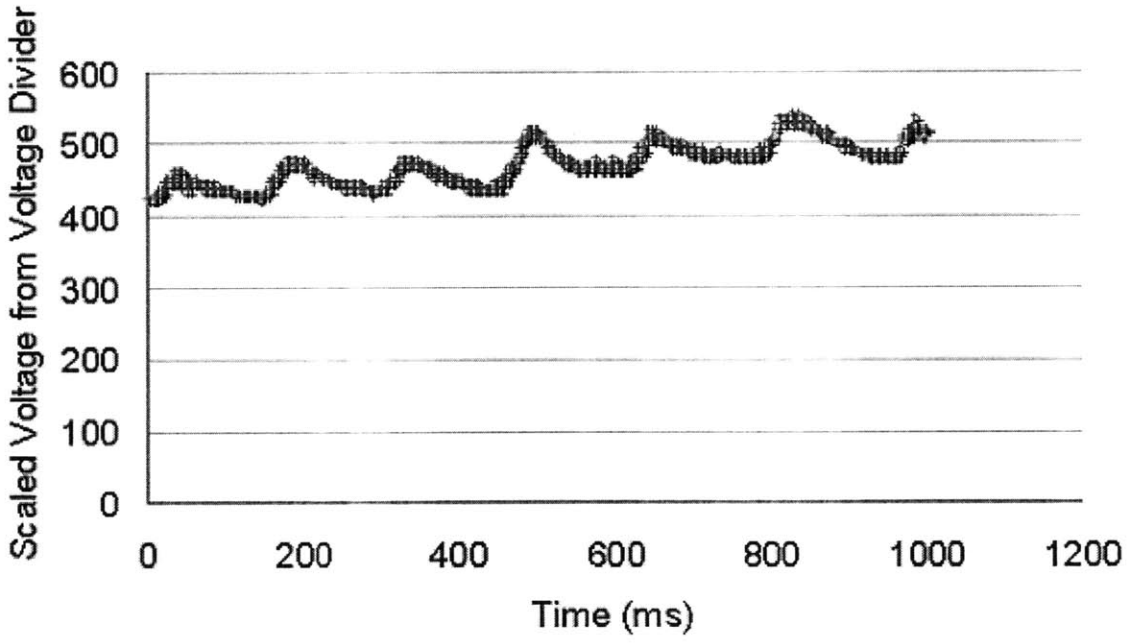

Figure 8. Results from a preliminary trial with a manual setup of the particle size distribution measurement device. A foot pump was used as a pressure source.

Figures 9a and 9b show the laser beam being interrupted by nebulized water during a test. The steady plateau at the beginning and end of both figures shows the constant voltage value reported by the photoresistor when the laser is aimed at it and no aerosol is present. The decrease shown in both figures occurs when the compressor is powered on and causes nebulization.

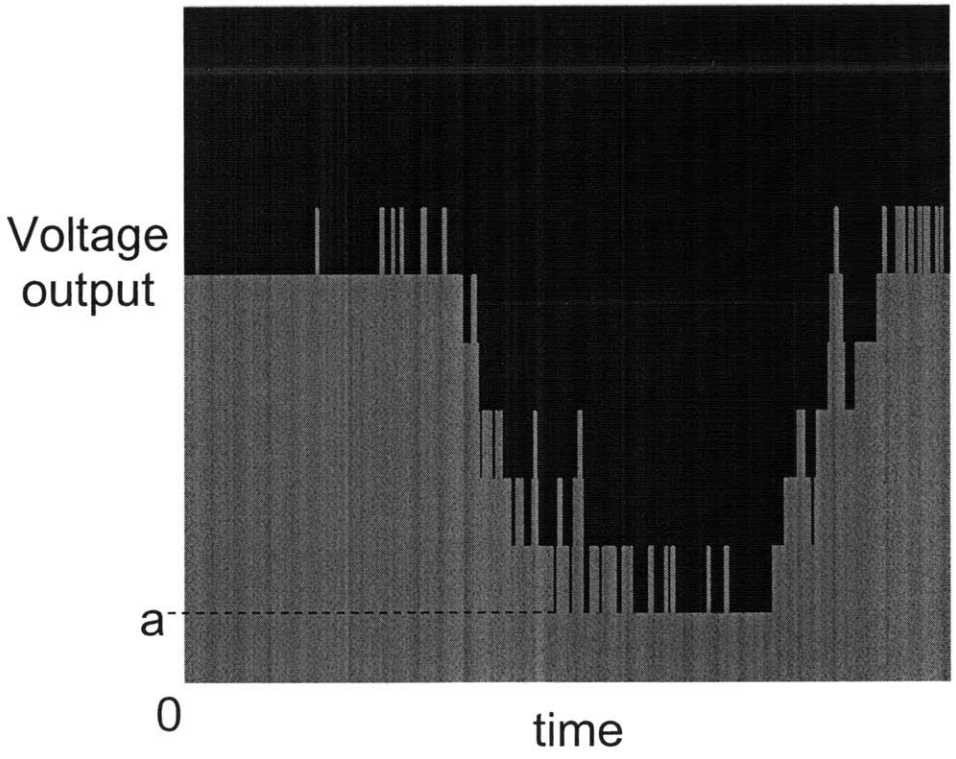

Figure 9a. Voltage reported by the photoresistor over time. The decrease occurs when aerosol interrupts the laser beam. The pressure input was from the compressor setup.

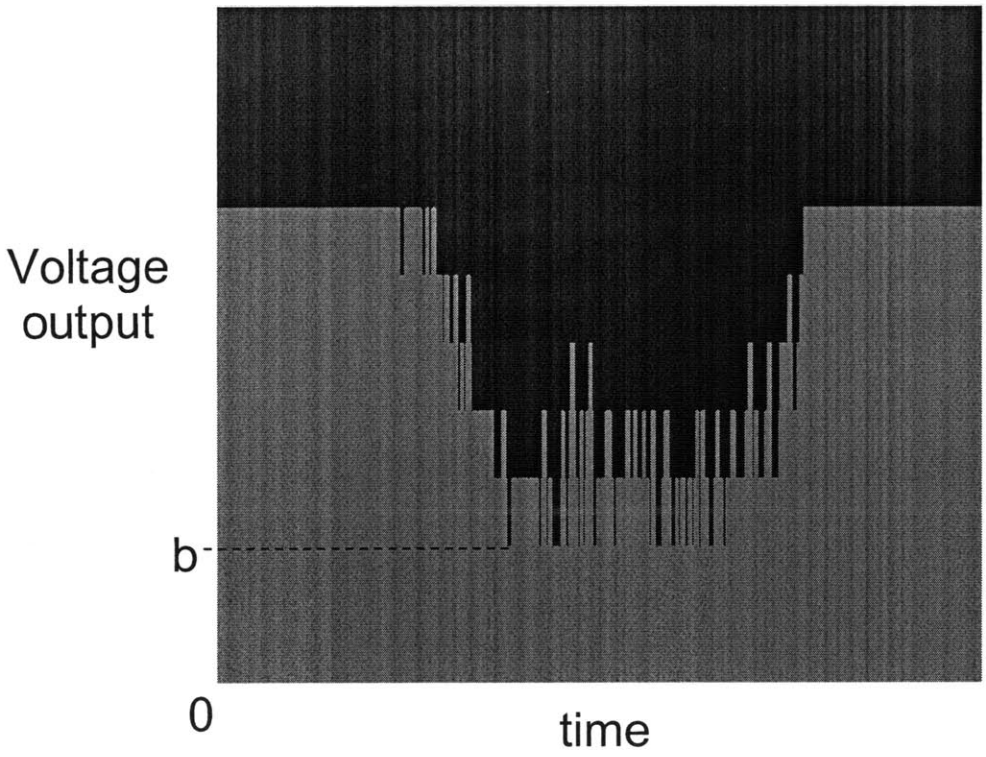

Figure 9b. A different set of results for the same experiment described in Figure 9a.

These initial readings allow for a qualitative comparison between different particle size distributions. Compared to voltage ouput *a* in Figure 9a, voltage output *b* in Figure 9b is larger. This suggests that less of the laser beam was blocked during the second trial, because the voltage output measured was closer to the initial value. It can be inferred that the overall particle size distribution for the trial shown in Figure 9b was the smaller of the two, and it can be hypothesized for future measurements that the larger the voltage output recorded, the smaller the particle size distribution of the aerosol.

4. Future of Aerovax

4.1 Optimization of Powdered Measles Vaccine

Using the system constructed, ambient properties, nebulizer geometry, and vaccine reconstitution can be adjusted to find the ideal parameters for producing an aerosol particle size of 3 μm to 5 μm.

To simulate the climates where Aerovax will operate, the ambient temperature and relative humidity of the experimental area will be altered. The temperature can be varied with the Amana Dryer Element Restring kit[6], which is a simple heating coil. The relative humidity will be modified with a Honeywell QuietCare Cool Moisture Humidifier (HCM650)[6].

This initial setup allows for switching to other geometries of jet nebulizers. While it is recognized that nebulizer shape has an effect on the properties of the aerosol it produces, there has been little research done that ties different aspects of the nebulizer geometry to attributes of the aerosol output. Nebulizer elements of interest include the jet nozzle diameter, the baffle surface area, and the distance between them[6], shown in Figure 10.

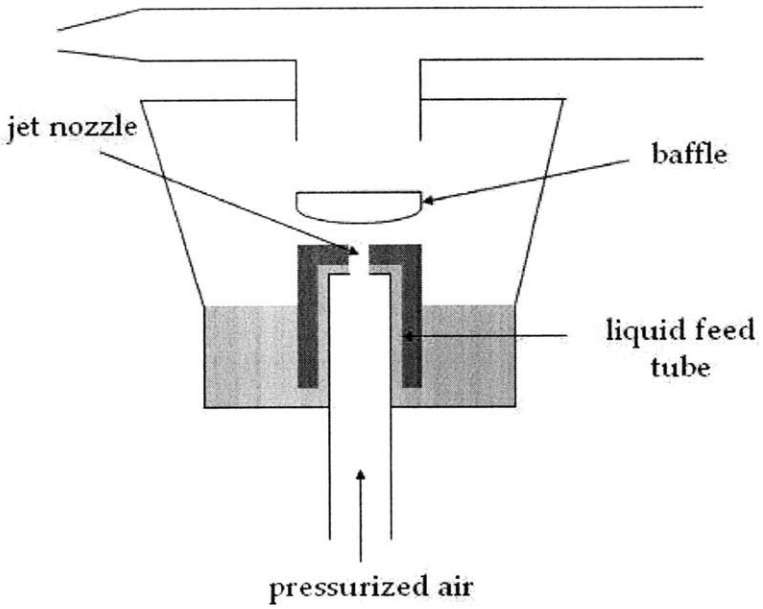

Figure 10. Diagram of a standard jet nebulizer[6].

Future iterations of the Aerovax experimental setup will include different forms of the jet nebulizer that vary the specified properties. An attempt will be made to connect any changes noted in the aerosol particle size distribution or nebulizer temperature to features of the nebulizer geometry[6].

If the current particle distribution measurement device proves too primitive, it will be replaced by the Malvern Spraytec Laser Diffraction instrument. The pressure transducer will also need to be replaced by a model that offers a higher range of pressure outputs (see Section 3.1), as the current model does not reach the maximum pounds per square inch needed for thorough aerosolization experimentation.

Reconstitution is the process by which the powder form of the vaccine is prepared for aerosolization. To guarantee proper sterility, potency, and overall safety, the correct type and amount of diluent must be used to dissolve each kind of vaccine[2]. Buffer solution and solute concentration are the defining variables of reconstitution. It should be noted that the solute, or vaccine, concentration tends to increase with time. This is caused by water evaporation from the solution at a rate faster than that of nebulization[9]. The amount of buffer solution and solute influences the vaccine's surface tension, pH, and viscosity. Each of these factors then has an effect on droplet formation during nebulization[6]. Surface tension, pH, and viscosity can be varied independently of each other in future Aerovax experiments dealing directly with the measles vaccine, as laid out by Nicholson[6].

Ultimately, the Aerovax experimental setup will contain a self-correcting feedback loop such that the parameters automatically adjust for the best possible outcome, which will depend on the aerosol fingerprint for the particular vaccine being tested. The optimization loop will vary pressure in an attempt to attain the most favorable particle size distribution for lung deposition in different conditions.

4.2 Extension to Other Vaccines

While Aerovax is currently intended for use with the measles vaccination, there is nothing prohibiting the expansion of its use to study other types of vaccines. Ultimately, a goal of the project is to have Aerovax operable with as many vaccines as possible. Measles was the chosen vaccine of focus for the first run of Aerovax because of its pervasiveness in the developing world. However, there are many other common diseases

that can be prevented with appropriate vaccination. These include, but are not limited to, pertussis, tetanus, polio, hepatitis (A and B), influenza, meningococcus, diphtheria, and yellow fever.

Parameters would need to be redefined for each vaccine; for example, certain ambient temperature and relative humidity values might influence a new vaccine's particle size distribution differently than they influenced the particle size distribution of the measles vaccine. The setup design and measurement techniques could remain the same, although preparation of the vaccine assays would need to be considered individually based on the unique biochemistry of each virus.

5. Conclusion

Vaccination by injection is not a viable solution for the mass measles immunization that is necessary in the developing world; an alternative method is needed. Inhalable vaccination has been verified as safe and equally effective as vaccination by subcutaneous injection. Aerovax will extend vaccination coverage to those for whom safe vaccination is not currently a possibility.

The setup presented in this thesis provides a foundation for future experimentation to determine the best method of delivering the aerosolized vaccine to the patient. The working model provides an input pressure that can be varied, nebulizer temperature measurement, and a device to gauge particle size distribution.

This setup is just the beginning of the optimization process. Future experiments will define the aerosol fingerprint[6] for the measles vaccine and for other selected vaccines. This research is the first attempt to optimize aerosolized vaccines across

multiple parameters, and will ultimately lead to the development of a vaccination device appropriate for use in the developing world.

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